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CONGLUTINATION TEST FOR THE DIAGNOSIS OF GLANDERS

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REVIEW OF PREVIOUS SERODIAGNOSTICAL WORK

Within the last decade rapid strides have been made in the diagnosis of infectious diseases by means of the various serological tests. In veterinary medicine glanders has perhaps received the most attention of those interested in serological work, owing in part to its prevalence the world over, the economic importance of its early and sure diagnosis, and its great menace to the equine and allied species, as well as to man. The first steps in the serodiagnosis of glanders were made in 1896, when M'Fadyean (5)[1] called attention to the possibilities of the employment of technic similar to the Widal agglutination reaction for typhoid fever. The agglutination test, however, was not generally adopted until the method was perfected by Schütz and Miessner (11), whose work was published in 1905.

In 1908 Schütz and Schubert (12) published their important work on the diagnosis of glanders by means of the complement-fixation test. By the simultaneous employment of both the agglutination and complement-fixation tests, the serodiagnosis of glanders was firmly established as a most reliable means of determining the presence or absence of glanders infection.

In 1912 Pfeiler and Weber (8) published a report on a new method for the serodiagnosis of glanders, the conglutination test, utilizing the phenomena that occur when certain definite amounts of fresh normal horse serum, inactivated cow serum, and washed red blood corpuscles of the sheep are brought together, resulting in a clumping or agglutination of the red blood cells followed by more or less hemolysis. This test has been employed in the diagnosis of syphilis and dysentery in man, while Pfeiler and Weber (7), Stranigg (14), Waldman (16), Anderson (1),

[1] Reference is made by number to "Literature cited," p. 74-75.

Michin (6), and others have studied the reaction for the diagnosis of glanders very closely, large numbers of serums being tested simultaneously by the agglutination, complement-fixation, and conglutination tests. All these investigators reported very favorably on the conglutination test, their conclusions being that it was a valuable addition to the serum tests for the diagnosis of glanders and should be employed in conjunction with the agglutination and complement-fixation tests. Schütz and Waldman (13) reported good results with the conglutination test, and also a modification of this test in testing the serum of the mule and ass.

In this country work has been done on the conglutination test by Fitch (4) in the diagnosis of glanders and by Wehrbein (17) in the diagnosis of dourine.

The phenomena on which the conglutination test is based were discovered through the work of Ehrlich and Sachs (3), who found, by combining fresh normal horse serum, inactivated cow serum (heated at 56° C. for one-half hour), and the washed red blood corpuscles of the guinea pig, that after a certain time the guinea-pig corpuscles were agglutinated and to a certain extent hemolyzed.

Bordet and Gay (2) and later Streng (15) studied this phenomenon and explained the reaction by stating that in fresh normal horse serum, besides alexin or complement, there is a normal sensitizer or amboceptor which has an affinity, on the one hand, for the alexin in the horse serum and on the other hand for the blood corpuscles. When this affinity has been satisfied the blood corpuscles are capable of taking up the alexin. After the corpuscles are alexinized they are then capable of being acted upon by a certain substance in the cow serum, a colloidal substance, termed by Bordet (2) "colloïde de bœuf," and later conglutinin, which has the property of strongly agglutinating and hemolyzing the blood corpuscles. The term "conglutination" was applied to this reaction.

Streng (15), experimenting with a large number of serums of various animals to determine the presence of conglutinin, found that this substance was not peculiar to cow serum alone, as he was able to demonstrate it in the serums of all ruminants, failing only in one instance with goat serum, and he attributes this failure to the fact that the goat used was quite young. The source of the complement is also immaterial, as serums from rabbits, guinea pigs, and man work equally well. Blood corpuscles from the guinea pig, sheep, or goat can be used with good results; with the sheep and goat, however, little hemolysis takes place, although the corpuscles are very strongly agglutinated.

Great similarity exists between the complement-fixation and conglutination tests, as the latter is also a complement deviation, with the difference that instead of hemolysis as an indicator, an agglutination of the red blood corpuscles takes place.

TECHNIC OF THE CONGLUTINATION TEST

Five factors enter into the conglutination reaction as in complement fixation: (1) Serum to be tested, (2) antigen, (3) complement (horse serum), (4) inactivated cow serum (conglutinin), and (5) sheep blood corpuscles.

A negative reaction is indicated by conglutination (clumping) of the red blood cells, the corpuscles being spread out in the bottom of the tube with a distinct ragged border. On shaking the tube the large clumps of red blood cells are very easily seen. Some hemolysis may take place, in which case the fluid is stained. In a positive reaction no conglutination takes place, the blood cells settling to the bottom of the tube in a mass, piling one on top of the other, with a very sharp regular outline, leaving an absolutely clear fluid above. The corpuscles settle to the bottom of the tube more slowly than in a negative case, as in the negative case the corpuscles are clumped which makes them settle more rapidly than the individual cells. On shaking the tube in a positive (+) case the blood corpuscles leave the bottom of the tube with a characteristic whirling, the fluid assumes its original opacity, and no clumping of the corpuscles is observed.

Certain differences in the technic of the conglutination test exist among the several investigators, the principal ones being (A) to add all the ingredients at once in the following order: suspected serum, antigen, horse complement, inactivated cow serum, and sheep blood, to allow the tubes to stand at room temperature, and to read the results in two to four hours; or (B) to add suspected serums, antigen, and complement, incubating for one hour at 37° C., then to add the cow serum and sheep-blood corpuscles, returning the ingredients to the incubator and reading the results from in four to nine hours. The technic employed in the work of the writer is similar to that employed by Pfeiler and Weber (9), with some few changes, and consists in combining all the reagents one after the other, allowing them to stand at room temperature, and reading the results in two hours.

The best results were obtained by following this technic:

Preparation and titration of conglutination system—

1. Fresh normal horse serum (complement).
2. Inactivated cow serum (conglutinin).
3. Washed sheep-blood corpuscles.

1. HORSE SERUM, OR COMPLEMENT.—A sufficient quantity of blood is drawn from the jugular vein of a normal horse. The blood is allowed to clot and after a time the serum is poured off. It is important that the serum be absolutely clear and free from red cells. The horse complement should be used fresh—that is, the same day on which it is drawn—as it can not be used after 24 hours.

2. COW SERUM.—This is obtained by bleeding a normal cow from the jugular vein into a sterile flask containing glass beads. The blood is defibrinated and then centrifuged and the clear serum drawn off. This should also be absolutely clear and free from red cells. The cow serum is then heated at 54° C. for half an hour, after which it is carbolized 0.5 per cent and stored in dark-colored bottles in the ice chest. The cow serum can be used for four to six weeks.

3. SHEEP RED BLOOD CORPUSCLES.—A sheep is bled from the jugular vein into a sterile flask containing glass beads. The blood is defibrinated and then centrifuged, the serum drawn off, and the corpuscles washed four times with a salt solution (0.85 per cent).

TITRATION OF THE COW SERUM

Constant amounts of horse serum (0.1 c. c.) and sheep blood (0.1 c. c. of a 5 per cent suspension) are used. It is now necessary to determine the smallest amount of cow serum that will cause conglutination of 0.1 c. c. of a 5 per cent suspension of sheep-blood corpuscles with 0.1 c. c. of fresh normal horse serum. Tubes of 13 mm. diameter and 9.5 cm. length are used in the test.

The results of the titration are given in Table I.

TABLE I.—*Titration of cow serum*

Tube No.	Complement.[a]	Salt solution.[b]	Inactivated cow serum.[c]	Sheep-blood corpuscles.[d]	Result.[e]
	C. c.	*C. c.*	*C. c.*	*C. c.*	
1	0.1	0.7	0.1	0.1	+
2	.1	.3	.05 =0.5	.1	+
3	.1	.6	.02 =0.2 } 1:10	.1	+
4	.1	.7	.01 =0.1 }	.1	+
5	.1	.3	.005=0.5 }	.1	±
6	.1	.6	.002=0.2 } 1:100	.1	±
7	.1	.7	.001=0.1 }	.1	−
f 8	.1	.8	.....	.1	−
f 9	.....	.08	.1	.1	−
f 10	.....	.09	.....	.1	−

[a] Fresh normal horse serum.
[b] 0.85 per cent.
[c] Cow serum inactivated at 54° C. for half an hour and carbolized 0.5 per cent.
[d] Five per cent suspension of washed sheep-blood corpuscles.
[e] + = conglutination; ± = partial conglutination; − = no conglutination.
[f] Control.

In Table I the smallest quantity of cow serum producing complete conglutination of 0.1 c. c. of a 5 per cent suspension of sheep-blood cells in 2 hours is represented by tube 4 as 0.01 c. c. Twice this amount is used in the test, making the titer of the cow serum 0.02 c. c. The cow serum need only be titrated once, provided the complement is derived from the same horse each time and the same sheep is always used to obtain the red-blood cells. The conglutination system is now titrated (Table II).

TABLE II.—*Titration of the conglutination system*

Tube No.	Complement.[a]	Inactivated cow serum.[b]	Sheep-blood corpuscles.[c]	Result.[d]
	C. c.	*C. c.*	*C. c.*	
1	0.1	Cow serum 0.02 + Salt solution 0.78 = 0.8	0.1	+
[e] 2	0.1	Cow serum ... + Salt solution 0.8 ...	.1	−
[e] 3	...	Cow serum 0.02 + Salt solution 0.78 = 0.8	.1	−
[e] 4	...	Cow serum ... + Salt solution 0.8 ...	.1	−

[a] Fresh normal horse serum.
[b] Inactivated cow serum plus salt solution.
[c] Five per cent suspension of washed sheep corpuscles.
[d] +=conglutination; −=no conglutination.
[e] Tubes 2, 3, and 4 are controls on the complement, cow serum, and the sheep blood.

PREPARATION AND TITRATION OF ANTIGEN

The same antigen that was used in the complement-fixation test was used in the conglutination test and is prepared as follows:

Bacillus mallei is grown for 24 to 48 hours on 5 per cent glycerin agar, with the reaction adjusted to + 2 per cent acid. When the growth has become luxuriant, it is washed off with distilled water carbolized 0.5 per cent and then heated at 62° C. for 3 hours. It is then placed in an ice chest for four or five days, after which it is centrifuged for 2 hours at a speed of 2,100 revolutions per minute. The supernatant fluid, which is quite clear, is poured off and represents the antigen.

A preliminary titration of the antigen is now made with the conglutination system. Ten tubes are used, and the ingredients are added in the following order: Antigen, horse complement, inactivated cow serum, and sheep blood. The tubes are shaken and allowed to stand at room temperature for 2 hours, when the results are read (Table III).

TABLE III.—*Preliminary titration of the antigen*

Tube No.	Antigen.[a]	Complement.[b]	Inactivated cow serum.[c]	Sheep-blood corpuscles.[d]	Result.[e]
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*
1	0.03	0.1	0.8	0.1	+
2	.05	.1	.8	.1	+
3	.08	.1	.8	.1	+
4	.1	.1	.8	.1	+
5	.15	.1	.8	.1	+
6	.2	.1	.8	.1	+
7	.25	.1	.8	.1	±
8	.3	.1	.8	.1	±
9	.35	.1	.8	.1	−
10	.4	.1	.8	.1	−

[a] Dilution of stock antigen 1:10.
[b] Fresh normal horse serum.
[c] Inactivated cow serum plus salt solution.
[d] 0.1 c. c. of a 5 per cent suspension of sheep-blood corpuscles.
[e] +=conglutination; ±=partial conglutination; −=no conglutination.

This titration indicates the largest amount of antigen which does not interfere with conglutination. After this preliminary titration the antigen is titrated against known positive and negative glanders serum, with varying amounts of antigen, the investigator being guided in these amounts by the results of the preliminary titration, so as not to use quantities so large that interference with conglutination occurs (Table IV).

TABLE IV.—*Titration of antigen against known positive and negative glanders serum*

Tube No.	Glanders serum.[a]		Antigen.[b]	Complement.[c]	Inactivated cow serum.[d]	Sheep-blood corpuscles.[e]	Result.[f]
	Positive.	Negative.					
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	*C. c.*	
1	0.1	.....	0.03	0.1	0.8	0.1	−
2	.1	.....	.05	.1	.8	.1	−
3	.1	.....	.08	.1	.8	.1	−
4	.1	.....	.1	.1	.8	.1	−
5	.1	.....	.15	.1	.8	.1	−
6	.1	.....	.2	.1	.8	.1	−
7	.1	.....	.....	.1	.8	.1	+
1	.....	.1	.05	.1	.8	.1	+
2	.....	.1	.08	.1	.8	.1	+
3	.....	.1	.1	.1	.8	.1	+
4	.....	.1	.15	.1	.8	.1	+
5	.....	.1	.2	.1	.8	.1	±
6	.....	.1	.3	.1	.8	.1	−
7	.....	.1	.....	.1	.8	.1	+
8	.....	.....	.....	.1	.8	.1	+

[a] Known positive and negative glanders serums.
[b] Glanders-bacilli extract.
[c] Fresh normal horse serum.
[d] Inactive cow serum diluted so that each 0.8 c. c. contains 0.02 c. c. of cow serum.
[e] Five per cent suspension of sheep-blood cells.
[f] +=conglutination; ±=partial conglutination; −=no conglutination.

Tube 7 is a control on the positive and negative serum, while tube 8 controls the conglutination system. The antigen is used as strong as possible; that is, the largest amount inhibiting conglutination in the positive case is used, provided that twice this amount does not interfere with conglutination in the negative case. In Table IV the titer of the antigen would be represented by 0.08 c. c. The antigen should be titrated each time a test is made.

INACTIVATION OF TEST SERUM

The serum to be tested is inactivated at 58° C. for half an hour, and should be clear and in good condition. Occasionally, however, in the quantities used in the test (0.05 and 0.1 c. c.) the serum controls will show an inhibition of conglutination. This occurs with serum that is heavily carbolized, decomposed, or with clear serum that has been kept at room temperature for a considerable period.

In a number of instances this serum can be utilized by titrating the serum against the conglutination system by using in the test proper the largest amount of serum not interfering with conglutination (Table V.).

TABLE V.—*Titration of test serum*

Tube No.	Serum.[a]	Complement.[b]	Inactivated cow serum.[c]	Sheep-blood corpuscles.[d]	Result.[e]
	C. c.	*C. c.*	*C. c.*	*C. c.*	
1	0.01	0.1	0.8	0.1	+
2	.02	.1	.8	.1	+
3	.03	.1	.8	.1	+
4	.04	.1	.8	.1	±
5	.05	.1	.8	.1	−
6	.06	.1	.8	.1	−

[a] Test serum.
[b] Fresh normal horse serum.
[c] Inactivated cow serum diluted 2 : 78.
[d] Five per cent suspension of sheep-blood corpuscles.
[e] +=conglutination; ±=partial conglutination; −=no conglutination.

With the above serum the largest amount not interfering with conglutination (0.03 c. c.) would be used in the test.

THE TEST PROPER

Various investigators have recommended the use of different quantities of the serum to be tested, ranging from 0.05 to 0.2 c. c. In this work 0.05 and 0.1 c. c. were found to be the most suitable. Four tubes are used to each serum sample to be tested, the first two tubes containing 0.05 c. c. of serum (the first tube receiving antigen, the second being a control on the serum) and the third and fourth tubes containing 0.1 c. c. (the third receiving antigen and the fourth serving as a control on 0.1 c. c. of the serum).

TABLE VI.—*Results of the conglutination tests proper*

POSITIVE CASE

Tube No.	Suspected serum.[a]	Antigen.[b]	Complement.[c]	Inactivated cow serum.[d]	Sheep-blood corpuscles.[e]	Result.[f]
	C. c.	*C. c.*	*C. c.*	*C. c.*	*C. c.*	
1	0.05	0.08	0.1	0.8	0.1	−
2 *g*	.05	.....	.1	.8	.1	+
3	.1	.08	.1	.8	.1	−
4 *g*	.1	.....	.1	.8	.1	+

NEGATIVE CASE

5	.05	.08	.1	.8	.1	+
6 *g*	.05	.....	.1	.8	.1	+
7	.1	.08	.1	.8	.1	+
8 *g*	.1	.....	.1	.8	.1	+

[a] Serum to be tested.
[b] *Bacillus mallei* extract.
[c] Fresh normal horse serum.
[d] Inactivated cow serum diluted 2 : 78.
[e] Five per cent suspension of sheep red-blood cells.
[f] No conglutination=positive case; +=conglutination=negative case.
[g] Tubes 2 and 4 and 6 and 8 are controls on the serum, and should not interfere with conglutination.

The advantage in using the two quantities of serum is found in those serums which inhibit conglutination in quantities of 0.1 c. c. in the serum control, whereas no such inference is noted with the smaller quantity (0.05 c. c.), in which case a diagnosis can be made on this amount, thus saving the necessity of a retest with smaller amounts of serum. Also occasionally a paradoxical reaction will be observed in which a serum gives a positive reaction with 0.05 c. c. of serum and a negative reaction with 0.1 c. c. These cases, however, are rare.

The results of the test are given in Table VI.

RESULTS OF COMPLEMENT-FIXATION AND CONGLUTINATION TESTS ON HORSE SERUMS

Four hundred and fifty-six samples of serums from horses and mules were tested by both the complement-fixation and conglutination tests. Unfortunately in a number of cases no post-mortem data could be obtained, but those samples in which positive results were obtained to both tests were in the majority of cases from animals giving a positive or suspicious reaction to the ophthalmic mallein and were undoubtedly affected with glanders (Table VII).

TABLE VII.—*Comparative tests of 341 serums from horses*

Number of samples.	Complement fixation.	Conglutination.	Post-mortem.	Remarks.
134	134+	134+	69+	Post-mortem report could not be obtained on other samples.
174	174−	174−	.....	
3	3+	3−	2+	No post-mortem report on third obtainable.
7	7−	7+	1+	Post-mortem data obtained in only 1 case, typical glanders lesions (chronic).
5	5?	5−	.....	
9	9?	9+	.....	No post-mortem report available.
9	9−	9?	.....	

Total number of samples tested=341; positive to complement fixation=137; positive to conglutination test=150; negative to complement fixation=190; negative to conglutination=182; doubtful to complement fixation=14; doubtful to conglutination=9.

As will be seen from Table VII, 134 samples gave positive results to both the complement-fixation and conglutination test, and 174 samples gave negative results to both tests. Three samples gave positive results to complement fixation and negative to conglutination. Post-mortem reports were obtained on two of these cases, typical glanders lesions being found, which were of an acute nature. In this connection it should be mentioned that it has been determined by several investigators that in cases of recent infection the specific antibodies are detected sooner by complement-fixation than by the conglutination test.

Seven samples were negative to complement fixation and positive to conglutination, a post-mortem report being received in only one case,

typical glanders lesions of a chronic nature being found. Five samples were questionable to complement fixation and negative to conglutination. Nine samples were questionable to complement fixation and positive to conglutination; no post-mortem reports were obtained. Nine samples were negative to complement fixation and questionable to conglutination.

RESULTS OF COMPLEMENT-FIXATION AND CONGLUTINATION TESTS ON MULE SERUMS

One disadvantage of the complement-fixation test is found when testing mule serums and those horse serums which possess nonspecific complement-fixing bodies. Mule serum is noted for its anticomplementary action against guinea-pig complement. These anticomplementary bodies are as a rule, however, partially or wholly destroyed by inactivating the serum with salt solution for 35 minutes at a temperature of 62° C. However, at this temperature in certain serums bacteriolytic amboceptors are also destroyed, and unless the serum is rich in these amboceptors, a misleading or doubtful reaction will be obtained owing to the fact that the inactivation at 62° C. has destroyed such a number of amboceptors that sufficient do not remain to fix all of the complement. In these cases the reaction would be interpreted as doubtful or negative, whereas in reality it would be a positive case.

The conglutination test is of decided advantage in the testing of mule serums and horse serums possessing nonspecific complement-fixing bodies, as these serums possess no anticomplementary action against horse complement.

Inactivation of the serum can be accomplished by heating for half an hour at 58° C., thus also eliminating the danger of destruction of bacteriolytic amboceptors by too high an inactivation (Table VIII).

TABLE VIII.—*Comparative tests of 115 serums from mules*

Number of samples.	Complement-fixation.	Conglutination.	Post-mortem.	Remarks.
32	32+	32+	3+	
11	11−	11+	0	No post-mortem data.
8	8?	8+	3+	Typical glanders lesions.
12	12?	12−	0	Negative to ophthalmic-mallein test.
1	1−	1?	0	
51	51−	51−	.....	

Total number of samples tested=115; positive to complement-fixation test=32; positive to conglutination test=51; negative to complement-fixation=63; negative to conglutination=63; doubtful to complement-fixation=20; doubtful to conglutination=1.

Thirty-two samples gave positive results to both tests. Fifty-one samples gave negative results to both tests. Eleven samples were negative to complement-fixation and positive to conglutination; no data

could be obtained as to whether these samples were from glandered or nonglandered animals. Eight samples gave questionable results to complement-fixation and positive results to conglutination; of these post-mortem reports was obtained in three instances, typical glanders lesions being found. Twelve samples gave questionable results to the complement-fixation test and negative results to conglutination. These animals were apparently in perfect health, and had given negative results to the ophthalmic-mallein test. The questionable results to the complement-fixation test in these cases were regarded as being nonspecific. One sample gave negative results to complement fixation and questionable to conglutination.

SUMMARY

(1) The conglutination test for glanders is a specific complement-deviation reaction.

(2) It should be used in conjunction with the complement-fixation and agglutination tests, as no one test is infallible.

(3) The conglutination test, as has been noted by others, is a more sensitive test than complement-fixation, and absolute accuracy in the technic is necessary to obtain good results, as a slight excess of any of the ingredients of the conglutination system will lead to a misleading reaction.

(4) The condition of the serum to be tested should be perfect in the conglutination test, although, if necessary, an unsatisfactory sample may be used by titrating it against the conglutination system.

(5) In the testing of mule serums and those horse serums possessing nonspecific complement-fixing bodies, the conglutination test is superior to the complement-fixation test.

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OCCURRENCE OF MANGANESE IN INSECT FLOWERS AND INSECT FLOWER STEMS

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INTRODUCTION

While working on chemical methods for the detection and estimation of powdered pyrethrum stems in insect powder (pulverized flower heads of *Chrysanthemum* (*Pyrethrum*) *cinerariaefolium* Vis.), the writers noted that Unger (14, 15)[1] proposed a method of determining whether a powder was strongly adulterated with stems by testing it for manganese. He stated that pyrethrum stems contained no manganese, while it was always present in the flowers. Thoms (13) found manganese in the ash of *C. leucanthemum* and *P. indicum*, both of which have been used to adulterate insect powder, and Jüttner and Siedler (7, p. 411) showed that pyrethrum stems from Dalmatia are not entirely manganese-free; but, inasmuch as none of the above investigators determined manganese quantitatively, it seemed worth while to test Unger's method more thoroughly. Furthermore, the present writers desired to test what effect the presence of manganese would have on the content of phosphoric acid.[2] They have devised a method[3] for the determination of powdered stems in insect powder, which is based partly on the difference in the phosphoric-acid content of the stems and flowers, and it was essential to know whether the presence or absence of manganese would affect this ratio.

The object in this investigation, therefore, has been twofold: (1) The exact estimation of manganese in the stems and flowers of *C. cinerariaefolium* from different countries in order to determine whether this element is constantly present in a greater degree in the flowers than in the stems, or vice versa, and (2) to determine what effect the presence of manganese has on the phosphoric-acid content.

PREVIOUS INVESTIGATIONS ON THE CONTENT OF MANGANESE IN PLANTS

It is not the purpose here to review all published work pertaining to the presence of manganese in various plants, but rather to refer to a few investigations bearing more directly on the problem with which the writers have been engaged.

[1] Reference is made by number to "Literature cited," p. 82.

[2] Kelley (8, p. 39) states that plants grown on highly manganiferous soil contain less phosphoric acid than the same plants grown on a soil containing only small amounts of manganese.

[3] Not yet published.

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In addition to the authors already cited, Sato (11) has observed that the ash of Japanese insect powder (made from flowers only) always contains manganese.

Hafner and Krist (3) examined 164 drugs qualitatively for manganese and found it in all but a very few. The flowers of the German chamomile (*Matricaria chamomilla* L.) and of the Roman chamomile (*Anthemis nobilis* L.), both of which have been used to adulterate insect powder, were found to contain manganese.

In regard to the distribution of manganese in the different parts of a plant, Saussure (12, p. 303) stated that the proportion of the oxids of iron and manganese in the ash increases as growth advances. Leaves of trees contain more of these oxids in the fall than in the spring, as is also the case with annuals. The seeds contain less than the stems.

Andreasch (1) found no manganese in the roots, stalk, or leaves of *Dianthus caryophyllus*, but there were unmistakable traces in the flowers. In the ash of the *Rosa remontana* a trace of manganese was found in the leaves, a distinct trace in the flowers, but none in the roots or stalk. This is the earliest recorded case of the finding of manganese in the flowers of a plant.

Pichard (10) held that manganese concentrates in the leaves and young shoots of a plant; or, in other words, in those parts of greatest vegetative activity.

Boname (2) always found more manganese in the leaves of sugar cane than in the stems, although the amount in both varied considerably from month to month.

Jadin and Astruc (4) stated that manganese is present in all plants. Of the same plant the aerial part contains more manganese than the subterranean part, from which they concluded that the chlorophyllic organs are richest in this element. These authors (6) also found more manganese in the old than in the young leaves, calculated on the fresh basis. On the dry basis the differences were of the same order but of less magnitude; on the basis of the ash, however, the order was reversed, there being more manganese in the ash of the young leaves. There were several exceptions to this rule.

Kelley (8, p. 35, 38; 9, p. 220–221) found that the distribution of manganese in the different parts of plants varies in different species. For example, the leaves of the pineapple contain 10 times as much manganese as the stalk, while, on the other hand, the stems of the peanut vine contain 10 times as much as the leaves. Usually, however, it is greatest in the leaves. In no instance was it found in greatest concentration in the seed.

If pyrethrum stems contained a much greater amount of manganese than the flowers, or vice versa, a determination of this element in an

insect powder would supply useful information as to the presence of powdered stems, provided, of course, that the variation in manganese content of either the stems or flowers was not too great.

EXPERIMENTAL WORK

For this investigation a number of representative samples of stems, "open" (mature) flowers, and "closed" flowers (buds) of both Dalmatian and Japanese origin were selected from a large number of samples that were on hand. In addition, a sample of buhach, made from the flowers of the *C. cinerariaefolium* grown in southern California, was tested. The number of samples could have been multiplied, but it is believed that the results obtained are thoroughly representative.

The method of analysis employed was as follows: Five gm. of material was ignited at a low temperature in an electric muffle, the ash dissolved in nitric acid, chlorids removed by the addition of silver nitrate and filtration, and the filtrate made up to convenient volume in a graduated flask. An aliquot of this was then oxidized with ammonium persulphate, and the amount of permanganate present determined by comparison with a standard solution of potassium permanganate in a Schreiner colorimeter (4, p. 117–118). Nitrogen and phosphoric acid were also determined.

The results of analyses are presented in Table I.

TABLE I.—*Analyses of Chrysanthemum cinerariaefolium* [a]

[Analyses on dried material; H_2O=5 to 7 per cent.]

Description.	Japanese.				Dalmatian.			
	Laboratory No.	Manganese.	Nitrogen.	Phosphoric.	Laboratory No.	Manganese.	Nitrogen.	Phosphoric.
		Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
Stems	22576	0.0077	1.05	0.38	12322	0.0059	0.84	0.14
	23773	.0176	.98	.34	12326	.0061	.70	.15
	24295	.0073	1.12	.39	12328	.0099	.84	.20
	24369	.0264	1.10	.41	12333	.0088	.86	.25
	24392	.0110	.98	.33	12336	.0110	.86	.....
	24394	.0110	.95	.35	14925	.0081	.80	.15
	24400	.0092	.77	.....	14931	.0077	.80	.17
	24401	.0132	1.02	.....	14946	.0077	.80	.24
	24402	.0110	.88	.....	15010	.0099	.71	.20
	24404	.0119	.84	.....	24190	.0066	.88	.35
	24409	.0092	.76	.....	24294	.0031	.55	.26
Average	.....	.0123	.....	.....	.....	.0077	.....	.....

[a] Buhach, made from flowers of *C. cinerariaefolium* grown in California (Lab. No. 26188); average=0.0092.

TABLE I.—*Analyses of Chrysanthemum cinerariaefolium*—Continued

Description.	Japanese.				Dalmatian.			
	Laboratory No.	Manganese.	Nitrogen.	Phosphoric.	Laboratory No.	Manganese.	Nitrogen.	Phosphoric.
		Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
Closed flowers	22577	0.0078	1.84	0.64	12325	0.0099	1.83	0.73
	22698	.0101	1.78	.70	12327	.0110	1.74	.62
	22944	.0159	1.96	.73	12334	.0044	1.65	.60
	22949	.0165	1.66	.....	14920	.0176	1.75	.74
	23772	.0187	1.61	.73	14929	.0119	1.65	.75
	24074	.0198	1.82	.....	15009	.0099	1.80	.74
	24116	.0137	1.73	.68	15136	.0044	1.87	.82
	24121	.0154	1.88	.....	22574	.0076	1.71	.....
	24298	.0220	1.80	.....	24314	.0008	1.67	.....
	.....	.....	.....	.....	24345	.0110	1.79	.....
	.....	.....	.....	.....	24347	.0088	1.63	.....
	.....	.....	.....	.....	24354	.0121	1.56	.....
	.....	.....	.....	.....	21208	.0154	1.75	.....
Average	.....	.0155	.....	.....	.....	.0096	.....	.....
Open flowers	.....	.....	.....	.....	12320	.0044	1.24	.53
	.....	.....	.....	.....	12323	.0066	1.26	.59
	.....	.....	.....	.....	12324	.0077	1.30	.53
	.....	.....	.....	.....	12329	.0092	1.31	.55
	.....	.....	.....	.....	12332	.0145	1.40	.56
	.....	.....	.....	.....	14924	.0088	1.31	.61
	.....	.....	.....	.....	14927	.0066	1.26	.55
	.....	.....	.....	.....	14930	.0051	1.22	.59
	.....	.....	.....	.....	14961	.0081	1.26	.59
	.....	.....	.....	.....	15008	.0090	1.24	.59
	.....	.....	.....	.....	15209	.0044	1.25	.59
	.....	.....	.....	.....	15270	.0088	1.25	.51
	.....	.....	.....	.....	15530	.0059	1.24	.46
Average	.....	.....	.....	.....	.....	.0076	.....	.....

It will be noted that manganese is generally present in *C. cinerariaefolium* from Dalmatia in both stems and "open" flowers to an equal amount, and to a slightly greater extent in the "closed" flowers. The analyses of samples from Japan showed that manganese is always present and in larger amount than in the Dalmatian samples. This is to be expected, because the soils of Japan are largely of volcanic origin and are known to contain large amounts of manganese. Stems of Japanese origin contain about three-fourths as much manganese as do the "closed" flowers from that country. "Open" flowers are not at present exported from Japan, so the writers have no available data on this product. The sample of buhach from California contains manganese. In fact, it is doubtful if any sample of insect powder is free from it.

The relation between the manganese, nitrogen, and phosphoric-acid content of stems and flowers of different degrees of maturity and different origin is brought out in Table II.

TABLE II.—*Analyses of Chrysanthemum cinerariaefolium. Average of all determinations, including many not reported in Table I* [a]

Description.	Manganese.	Nitrogen.	Phosphoric acid
	Per cent.	*Per cent.*	*Per cent.*
Japanese stems	0.0123 (11)	0.928 (12)	0.326 (12)
Dalmatian stems	.0077 (11)	.733 (61)	.192 (26)
Japanese closed flowers	.0155 (9)	1.813 (48)	.680 (15)
Dalmatian closed Flowers	.0096 (13)	1.759 (55)	.700 (19)
Japanese open flowers			
Dalmatian open flowers	.0076 (13)	1.267 (104)	.532 (94)

[a] The numbers in parentheses indicate the number of samples on which the determination in question was made.

It is seen that a high manganese content is accompanied by an increased amount of nitrogen and phosphoric acid rather than the reverse.

From the above results we may conclude that the determination of manganese in an insect powder is worthless for ascertaining whether it has been adulterated with pyrethrum stems. In a rough way a large amount of manganese indicates a product of Japanese origin, but the variation in the content of manganese is so great in pyrethrums from the same country that this test can not be applied with certainty to determine the geographic origin of an insect powder. In other words, the determination of manganese in a sample of insect powder supplies little or no useful information with respect to its purity, and Unger's method is shown to be valueless. Furthermore, the amount of manganese absorbed by plants of *C. cinerariaefolium* is so small as not to affect appreciably the ratio between the nitrogen and phosphoric acid of the stem and of the flowers,

SUMMARY

(1) Quantitative data are presented on the manganese content of stems, "open" flowers, and "closed" flowers of *Chrysanthemum cinerariaefolium* of both Dalmatian and Japanese origin.

(2) It is shown that the manganese content of both stems and flowers varies so much and is so little different in these two parts of the plant as to render valueless any method for estimating the amount of powdered stems in an insect powder from a determination of its manganese content.

(3) Pyrethrum of Japanese origin contains more manganese than that from other countries, owing presumably to the high manganese content of the volcanic soils of Japan.

(4) An increase in the manganese content of pyrethrum is accompanied by a slightly higher nitrogen and phosphoric-acid content.

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LIFE HISTORY OF MACROSIPHUM ILLINOISENSIS, THE GRAPEVINE APHIS

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INTRODUCTION

A brief note recording the alternation in food plants by this species was published by Baker and Turner in 1915 [1]. No complete account of the different forms, however, is at present in the literature. It seems best, therefore, in view of the economic importance of the insect, to record briefly the results of some more recent studies and to give an account of all the forms of the species. A brief popular account of the life history has been given by Quaintance and Baker.[2]

NAME

The insect under discussion was first described and named by Shimer [3] as *Aphis illinoisensis*. Later Thomas [4] described it as *Siphonophora viticola*. For many years Shimer's name was lost sight of, owing, no doubt, to the obscurity of its publication, and practically all of the references to the insect as a pest are found under the name "*viticola* Thos." Davis,[5] in listing the aphids of Illinois, called attention to Shimer's description and placed "*viticola* Thos." as a synonym of "*illinoisensis* Shimer."

OCCURRENCE

The species occurs quite abundantly on wild grapes (*Vitis* spp.) in the more southern parts of the country and often is quite destructive to cultivated varieties (Pl. 8, C, D). Specimens have been received from the District of Columbia, Georgia, Indiana, Maryland, Missouri, Mississippi, North Carolina, New Jersey, New York, Oklahoma, Pennsylvania, Texas, and Virginia. What may also be the same species was taken on grape by F. Noack, at Campinas, Brazil, during September, 1898.

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EGGS

The eggs are polished black and are laid upon the twigs of *Viburnum prunifolium*. They are usually placed most thickly close around the buds, although when the plants are thickly infested they may be distributed more evenly over the twigs. The eggs hatch sometimes during quite cold weather in the third week in March, but the insects produced from these first-hatched eggs are sometimes killed by frosts. Hatching continues until the early part of April.

STEM MOTHER

When first hatched, the stem mother is of a very dark greenish-brown color. The young aphids, on hatching, seek out the buds and begin feeding. Here they remain until the buds open, and when the flowers begin to open it is not uncommon to find them crowded down into the flower clusters. They feed upon the stems of the individual blossoms, and when the petals begin to show the presence of the aphids is often not noticeable. They also, however, feed upon the twigs and somewhat upon the leaves.

FIRST INSTAR.—Color dark greenish brown, the abdomen lighter than the other parts of the body. Appendages black, as is also the head; abdomen with a few rows of darker spots. Antennæ of three segments; segment III measuring 0.064 plus 0.08 mm. Cornicles very short and tuberculate, being about 0.016 mm. long.

Found feeding on the twigs of *Viburnum prunifolium*.

SECOND INSTAR.—Somewhat similar in color to the first instar, although of a more deep brown color rather than a greenish brown. Abdomen without apparent darker brown spots, but uniform brown. Appendages dusky to black. When first molted, the insects are an amber color. The empty skins are dusky. Antennæ of four or five segments; segment III, 0.08 mm.; IV, 0.06 mm.; V, 0.4 plus 0.08 mm.; cornicles, 0.05 mm.

Found in the same places as the first instar.

THIRD INSTAR.—Very similar in color to the second instar, although sometimes a little lighter. Antennæ of five segments; segment III, 0.112 mm.; IV, 0.08 mm.; V, 0.064 plus 0.112 mm.; cornicles, 0.064 mm.

Found in the same places as the earlier instars and also crowding down into the expanding buds.

FOURTH INSTAR.—Similar to the previous one in color. Antennæ of five segments; segment III, 0.16 mm.; IV, 0.08 mm.; V, 0.064 plus 0.112 mm.; cornicles, 0.112 mm.

FIFTH INSTAR (ADULT).—Color characters similar to those of the previous instars, but some of these forms are somewhat yellowish. Most of them, however, are a distinct reddish brown. Eyes deep brown, vertex dusky. Antennæ dusky, with the distal segment and the first two segments darker than the others. Legs similar in color to the antennæ, the tarsi and distal extremity of the tibiæ dusky. Cornicles dark. Antennæ of five segments; segment III, 0.208 mm.; IV, 0.112 mm.; V, 0.064 plus 0.128 mm.; segments, particularly the distal one, distinctly imbricated, but without sensoria other than the usual ones. Cornicles 0.144 mm. long, without a distal flange, but with faint imbrications.

Found on the leaves, twigs, and flowers of *Viburnum prunifolium* L.

SPRING MIGRANT

The spring migrants begin to appear in the second generation, although their number is not abundant until the third generation, and their production then gradually decreases for several generations. It is thus evident that the spring migration extends over a considerable period. It is at its height during the first week in May.

The spring migrants fly to wild grapes and to the grapes in vineyards. In Virginia, where the writer conducted his experiments, wild grapes often grew very close to the viburnums, and in some cases they even climbed over the viburnum bushes. In such cases migrants took grapes close at hand, although the vines in the viburnum bushes were not thickly infested. The migrants, however, are capable of rather extended flight, having been noted on grapes about a mile distant from the nearest viburnum bushes. It would appear that the first migrants developed take to the wild grapes in the vicinity of the viburnums, for the first migrant discovered upon the cultivated grapes was taken on April 28. This is considerably later than their first appearance. By May 1 this specimen had produced many young upon the grape, whereas the bulk of the migrants had not yet made their appearance.

FOURTH INSTAR (PUPA).—General color reddish brown, the wing pads, legs, and cornicles dark; eyes black. Antennæ yellowish brown with the distal segments dusky. Antennæ of six segments; segment III, 0.304 mm.; IV, 0.24 mm.; V, 0.208 mm.; VI, 0.096 plus 0.32 mm.; cornicles tapering, distinctly imbricated, and 0.336 mm. long. Length from vertex to tip of cauda, 1.36 mm.

FIFTH INSTAR (ADULT).—Color a deep reddish brown, appendages and cornicles darker, the tibiæ with a lighter median area; eyes black; wings with dusky veins and stigma. The antennæ are distinctly imbricated on all segments excepting I and II. Segment III armed with from 6 to 14 sensoria arranged in an uneven row on the basal three-fourths of the segment. In some cases the sensoria cover the entire segment. They are uneven in size, some being fairly large while others are quite small. The cornicles are somewhat tapering, very distinctly imbricated, and with distal extremity not distinctly flanged. Cauda about half the length of the cornicles. Length of fore wing, 2.4 mm.; length from vertex to tip of cauda, 1.328 mm. The relative lengths of the antennal segments and cornicles are given in Table I.

TABLE I.—*Lengths (in millimeters) of antennal segments and cornicles of spring migrant of Macrosiphum illinoisensis, with host, and number of sensoria on antennal segment III*

Date.	Host.	Segment III.	Number of sensoria on segment III.	Segment IV.	Segment V.	Segment VI.	Cornicles.
Apr. 28	Viburnum	0.432	13	0.336	0.272	0.096+0.32	0.368
Do.	do	.416	7	.352	.304	.112+.32	.4
Do.	do	.368	7	.32	.256	.112+.304	.352
Do.	do	.288	7	.32	.272	.112+.336	.352
May 1	Grape	.352	8	.304	.272	.096+.352	.336
May 4	do	.336	10	.288	.24	.096+.336	.416
May 5	do	.384	11	.336	.272	.112+.288	.368

SUMMER WINGLESS VIVIPAROUS FEMALE

The summer wingless forms (Pl. 9, B, C) occur very abundantly throughout the summer. They reproduce very quickly during the early summer, so that seven generations have often reached maturity by the 1st of July. The wingless forms reach maturity a day or two before the winged forms of the same generation, so that during the season many more wingless generations are produced upon grape than there are of winged generations. The wingless insects vary considerably in size and color, sometimes being a distinct brown, while other specimens are deep black. They also vary considerably in size, the darker, smaller individuals occurring later in the summer. The number of young produced daily ranges from 6 to 10, and the insects live for three weeks or more.

The summer wingless and winged forms do considerable damage to the vines in some sections. They attack the growing clusters, sometimes thickly covering them, and the feeding of the insects causes the berries to drop. Some vines at Vienna, Va., which the writer had under observation produced scarcely any fruit owing to the attacks of the aphids. The berries dropped while still green and ranging in size from that of a pea to that of a small cherry. The fruit stems in nearly every case had become somewhat withered owing to the continued feeding of the insects. The young growing shoots and leaves are also very abundantly attacked, and when the aphids are numerous growth is more or less retarded. The most important injury, however, appears to be that caused to the fruit clusters.

FIFTH INSTAR (ADULT) (Pl. 9, C).—General color a deep reddish brown. Antennæ with the basal two segments and the distal segment dusky, the remaining segments yellowish brown, dusky at the joints; eyes black; hind legs and cornicles black, femora of the middle and fore legs and a band about their tibiæ yellowish brown, remainder black; cauda dusky. Length from vertex to tip of cauda, 1.272 mm. Measurements of the antennæ and cornicles as follows (Table II):

TABLE II.—*Lengths (in millimeters) of the antennal segments and cornicles of the summer wingless vivipara of Macrosiphum illinoisensis*

Generation.	Segment III.	Segment IV.	Segment V.	Segment VI.	Cornicles.
Third	0.4	0.32	0.256	0.096+0.256	0.48
Sixth	.416	.32	.272	.112+.336	.496
Seventh	.416	.288	.272	.112+.336	.48
Eighth	.464	.368	.224	.096+.352	.56

INTERMEDIATE

Intermediates between the summer winged and the summer wingless forms have been found upon grape. These resemble more closely the winged form than they do the wingless one.

Thorax partially developed, some of the large wing muscles remaining. Wings represented by small pad-like structures about 0.4 mm. long. Antennæ and cornicles

practically like those of winged form, having a row of sensoria on segment III. Measurements of antennal segments average about as follows: Segment III, 0.432 mm. with about 9 sensoria; IV, 0.352 mm.; V, 0.256 mm.; VI, 0.096 plus 0.432 mm. Cornicles 0.495 mm.; length from vertex to tip of cauda, 1.92 mm.

SUMMER WINGED VIVIPAROUS FEMALE

Winged forms (Pl. 9, A, D) are produced in every generation from the second onward, but fewer winged line generations occur than wingless line generations. The winged forms show the same variations as the wingless ones, some specimens being large and light brown, while others are very small and black. The small dark forms occur during the hottest months of summer, while the large paler forms are usually met earlier in the spring. The winged forms produce an average of six young a day.

FIFTH INSTAR (ADULT) (Pl. 9, A).—General color dark brown. The color, however, varies greatly, some specimens being a deep black and others being almost of a yellowish color. Thorax black; eyes, hind legs, and cornicles black, middle and fore legs with yellowish bands around the segments and with the areas about the joints black. Wings hyalin, the veins and stigma dusky. Antennæ distinctly imbricated and with from 7 to 13 sensoria on segment III. Cornicles tapering, strongly imbricated, slightly curved and held outward from the body. Length of forewing, 2.72 mm.; length from vertex to tip of cauda, 1.44 mm. Relative lengths of antennal segments and cornicles given in Table III.

TABLE III.—*Lengths (in millimeters) of antennal segments and cornicles of summer winged vivipara of Macrosiphum illinoisensis, with the number of sensoria on antennal segment III*

Generation.	Segment III.	Number of sensoria on segment III.	Segment IV.	Segment V.	Segment VI.	Cornicles.
Third	0.416	11	0.32	0.304	0.144+0.384	0.4
Sixth	.4	8	.336	.272	.096+.32	.4
Eighth	.352	13	.336	.24	.096+.336	.384
Do	.272	7	.24	.208	.08 +.352	.32

FALL MIGRANT

The fall migrants are produced upon the grapes during the early part of October and are found upon the viburnum depositing young oviparous females during the second week in that month. They are somewhat different in general appearance from the summer forms, being lighter and smaller.

FIFTH INSTAR (ADULT).—General color reddish brown, rather pale as compared with the summer winged form. Thorax and sternal plate black; antennæ dusky; cornicles and hind legs black; anterior and middle legs yellowish brown, dusky at the joints; wings hyalin with dusky veins and stigma. Antennæ (Pl. 8, B) strongly imbricated, segments III, IV, and V with sensoria as indicated in Table III. Cornicles tapering, distinctly imbricated, and not enlarged on the distal tip. Cauda and anal plate black. Length of forewing, 2.7 mm.; length from vertex to tip of cauda, 1.44 mm.; measurements of antennal segments and cornicles given in Table IV.

TABLE IV.—*Lengths (in millimeters) of antennal segments and cornicles of fall migrant of Macrosiphum illinoisensis, with number of sensoria on antennal segments III, IV, and V*

Segment III.	Number of sensoria on segment III.	Segment IV.	Number of sensoria on segment IV.	Segment V.	Number of sensoria on segment V.	Segment VI.	Cornicles.
0.304	16	0.272	13	0.224	6	0.112+0.32	0.272
.384	28	.304	16	.288	8	.112+.352	.352
.304	17	.256	18	.224	7	.096+.336	.256

MALE

The males are produced a little later than the fall migrants, but can be found flying at the same time and may be taken on the viburnums in company with the fall migrants. Thus they are on the trees in many cases before the oviparous females are mature. They remain feeding upon the leaves until such time as copulation is possible.

FIFTH INSTAR (ADULT).—General color a pale reddish brown, often, however, of a distinct greenish color. Appendages dusky; eyes black; wings hyaline with dusky veins and stigma. Antennæ (Pl. 8, A) distinctly imbricated and segments III, IV, and V irregularly covered with very many small circular sensoria. Cornicles short, distinctly tapering and imbricated. Forewing 2 mm. long; length from vertex to tip of cauda, 1.2 mm. Relative lengths of antennal segments and cornicles given in Table V.

TABLE V.—*Lengths (in millimeters) of antennal segments and cornicles of male of Macrosiphum illinoisensis, with number of sensoria on antennal segments III, IV, and V*

Segment III.	Number of sensoria on segment III.	Segment IV.	Number of sensoria on segment IV.	Segment V.	Number of sensoria on segment V.	Segment VI.	Cornicles.
0.336	31	0.272	29	0.224	15	0.08+0.32	0.176
.352	30	.256	27	.208	15	.08+.336	.16
.288	38	.224	35	.176	19	.08+.336	.144
.288	35	.224	28	.176	17	.08+.352	.144

OVIPAROUS FEMALE

The oviparous female is a small, dark reddish aphis produced during the early part of October on the viburnum. It feeds upon the twigs and may be found until frost kills all the insects. Each oviparous female lays three to six eggs close about the buds or occasionally scattered along the twigs.

FIRST INSTAR.—General color greenish brown, almost uniform, the appendages, however, a little darker. Antennæ of four segments; segment III, 0.08 mm.; IV, 0.032 plus 0.112 mm.; cornicles very small and tubercle-like. Length from vertex to tip of cauda, 0.48mm.

SECOND INSTAR.—General color more reddish than the first instar, otherwise similar. Antennæ of four segments; segment III, 0.144 mm.; IV, 0.048 plus 0.16

mm. Cornicles cylindrical, often slightly tapering toward the tip. Eyes black. Length from vertex to tip of cauda, 0.64 mm.

THIRD INSTAR.—General color deep reddish brown with black eyes; antennæ dusky toward the tip and composed of five segments. Segment III, 0.112 mm.; IV, 0.064 mm.; V, 0.048 plus 0.192 mm. Cornicles 0.064 mm., somewhat tapering. Length from vertex to tip of cauda, 0.8 mm.

FOURTH INSTAR.—Color characters similar to those of the third instar, but somewhat lighter in general color. Antennæ of five segments. Segment III, 0.192 mm.; IV, 0.112 mm.; V, 0.08 plus 0.208 mm. Cornicles 0.96 mm., tapering, and rather broad. Hind tibia somewhat swollen. Length from vertex to tip of cauda, 0.96 mm.

FIFTH INSTAR (ADULT).—General color yellowish to reddish brown, eyes black; cauda and anal plate dusky; antennæ with the basal two segments and with the distal segment dusky; legs dusky, sometimes lighter on the median area of the tibiæ. Antennæ of six segments; segment III, 0.16 mm.; IV, 0.128 mm.; V, 0.128 mm.; VI, 0.08 plus 0.224 mm. All segments except the first two distinctly imbricated, no sensoria except the usual ones present. Cornicles distinctly tapering and imbricated, 0.096 mm. long, dusky in color; hind tibia somewhat swollen, 1.36 mm. long and armed with about 50 small, irregularly placed, circular sensoria. Length from vertex to tip of ovipositor, 1.44 mm.

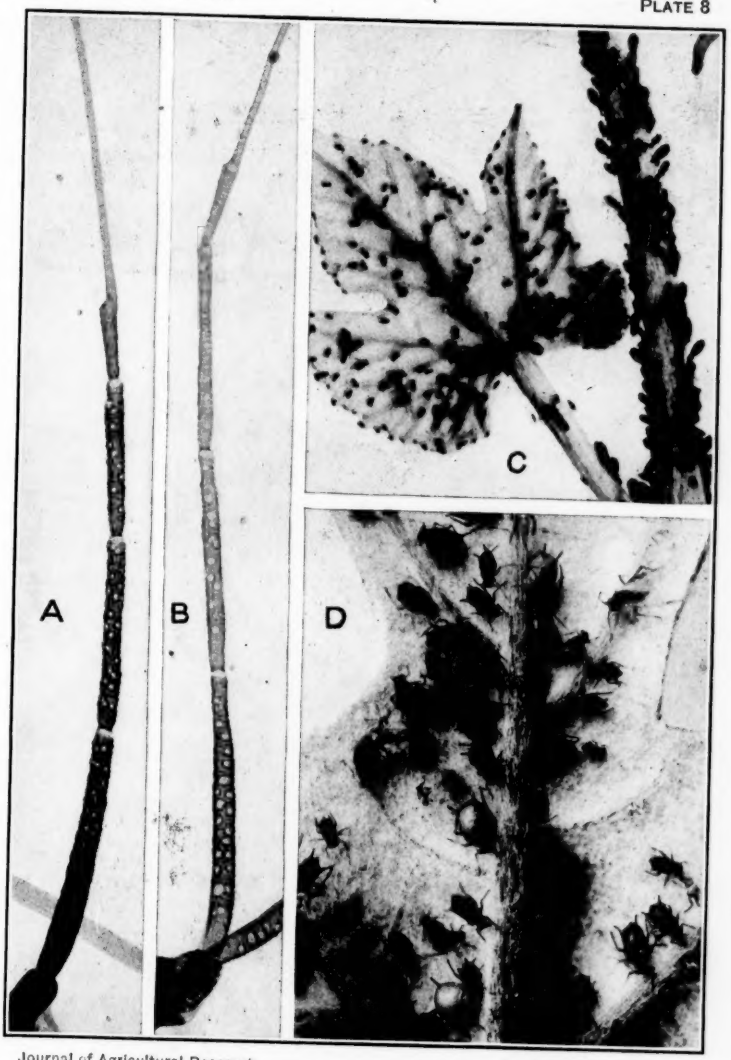
PLATE 8

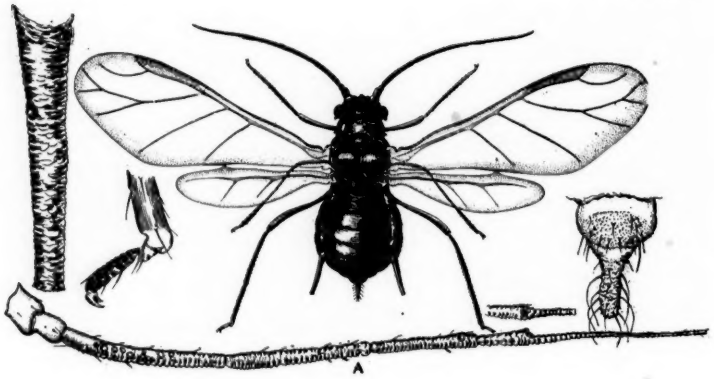
Macrosiphum illinoisensis:

A.—Antenna of male.
B.—Antenna of fall migrant.
C.—Colony on grape leaf.
D.—Colony on grape leaf, more enlarged.

Macrosiphum illinoisensis

PLATE 8

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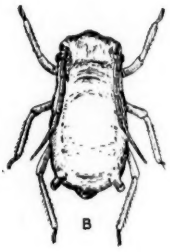
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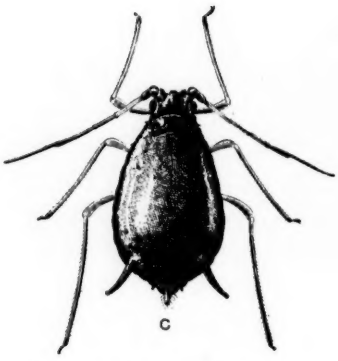
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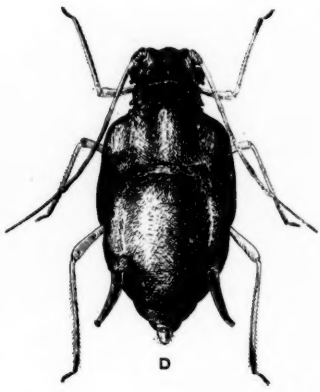
D

PLATE 9

Macrosiphum illinoisensis:

A.—Summer winged viviparous female, and details.
B.—Summer wingless viviparous female, first instar.
C.—Summer wingless viviparous female, fifth instar or adult.
D.—Pupa of summer winged viviparous female.